

**METHOD AND APPARATUS FOR THE PREPARATION OF BLOOD SAMPLES FOR AUTOMATED ANALYSIS**

**Patent number:** CA1129320  
**Publication date:** 1982-08-10  
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**Classification:**  
**- international:** G01N1/28  
**- european:**  
**Application number:** CA19790330583 19790626  
**Priority number(s):** CA19790330583 19790626

**Abstract of CA1129320**

A method for preparing blood samples containing red blood cells for automated analysis wherein the blood sample on a slide (20) is spun to create a monolayer of randomly distributed red blood cells (40, 42, 46). To inhibit cell morphology distortions from occurring during drying. The morphologies of the cells contained in the monolayer are preserved by a fixing agent after monolayer preparation but prior to drying when such distortions would otherwise develop. The method and apparatus are particularly useful for fixing red blood cells to prevent loss of or deformation of a central pallor which would be detrimental to a subsequent automated analysis. A vaporous fixing agent may be conveyed through a conduit (26) into a spinning chamber (14) enclosed by a cover (24) of a slide spinning apparatus to provide a fixing atmosphere therein to begin fixing the wet cell before they can dry.

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(11) (A) No. 1 129 320

(45) ISSUED 820810

(52) CLASS 150-11

(51) INT. CL. G01N 1/28<sup>3</sup>

(19) (CA) **CANADIAN PATENT** (12)

(54) METHOD AND APPARATUS FOR THE PREPARATION OF BLOOD  
SAMPLES FOR AUTOMATED ANALYSIS

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(21) APPLICATION No. 330,583

(22) FILED 790626

No. OF CLAIMS 10

330583

AUG10 1962

ABSTRACT

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A method for preparing blood samples containing red blood cells for automated analysis wherein the blood sample on a slide (20) is spun to create a monolayer of randomly distributed red blood cells (40, 42, 46). To inhibit cell morphology distortions from occurring during drying. The morphologies of the cells contained in the monolayer are preserved by a fixing agent after monolayer preparation but prior to drying when such distortions would otherwise develop. The method and apparatus are particularly useful for fixing red blood cells to prevent loss of or deformation of a central pallor which would be detrimental to a subsequent automated analysis. A vaporous fixing agent may be conveyed through a conduit (26) into a spinning chamber (14) enclosed by a cover (24) of a slide spinning apparatus to provide a fixing atmosphere therein to begin fixing the wet cell before they can dry.

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METHOD AND APPARATUS  
FOR THE PREPARATION OF BLOOD SAMPLES  
FOR AUTOMATED ANALYSIS

This invention relates to a method of, and an apparatus for, the preparation of blood cell specimens for diagnostic analysis. More specifically, the invention is particularly directed to the fixing or preservation of the morphology of cells for later automated analysis.

The present invention is particularly directed to fixing cells during a precise and critical step of sample preparation, such that their morphology is preserved for later analysis. Thus, the development of distortions encountered by other known methods of sample preparation having cells on a slide is reduced to a minimum. Although the present invention is not to be construed as limited to any particular kind of cell or sample, it will be described in connection with the preparation of red blood cells for automatic analysis on a rapid basis. One distortion noted heretofore in known methods of preparation of microscopic slides was that of the alteration of morphology of certain red blood cells, particularly the loss by certain cells of their central pallor, i.e., a thin central indented region in a cell, with the cell becoming more rounded and resembling spherocytes or other cells of a similar diameter or shape.

The diagnosis of various kinds of anemia is enhanced by accurate analysis of the sizes of the red blood cells and the quantification of red cell parameters, such as the Wintrobe indices of mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration. At the present time, such information is typically acquired by the use of a Coulter Counter which measures the number of red blood cells per cubic millimeter, the hemoglobin concentration, and the mean cell volume in a liquid flow system. In addition to this information, provided by the Coulter Counter, it would be most helpful to have an accurate analysis of the kinds of cells present in the sample in accordance with established hematological classifications, such as normocytes, macrocytes, target cells, microcytes, spiculed cells, hypochromic elongated cells, spherocytes, etc. A method of and apparatus for classifying individual cells into such classifications, i.e., subpopulations, and providing red blood cell parameters for each of the subpopulations, is disclosed in U.S. Patent No. 4,097,845 of James W. Bacus, entitled "A Method of and an Apparatus for Automatic Classification of Red Blood Cells".

The oldest and most common method of preparing microscopic slides for a manual evaluation under a microscope involves the wedge-slide technique in which a quantity of blood on a glass slide is wiped by a second slide along the surface of the first slide to produce a thin blood layer on the first slide. After the layer dries, which requires only a short time, the slide is dipped into a staining agent and then the operator views the slide under a microscope and visually analyzes the red cell population. In addition to being time consuming, the physical action of the wiping slide tends to distort the morphology of many of the cells. Typically, under the best of conditions, only a fraction of the surface area of the slide is suitable for analysis. This distortion renders this wedge technique unsuitable for automated red blood cell

analysis.

B ~~white~~ There has been developed, particularly for  
white blood cell analysis, a spinning technique for the  
formation of a monolayer of cells on a slide for later  
5 automated analysis of the white blood cells. Es-  
sentially, such an apparatus spins a slide and the blood  
placed thereon in the plane of the surface on which the  
blood rests with the excess blood being hurled outward-  
ly from the slide surface. Surface tension and/or  
10 other forces retain a monolayer of blood upon the slide.  
For white blood cell work, the cells in the monolayer  
are allowed to dry and then are stained with a Wright's  
stain (or other stain) and analyzed automatically such  
as disclosed in U.S. Patent No. 3,883,852.

15 While the drying of the white blood cells has  
not produced a substantial distortion from a white blood  
cell analysis standpoint, it has been found that the  
drying of the red blood cells produces undesirable types  
of distortions, particularly a loss of central pallor  
20 for many of the red blood cells as they dry. It is not  
entirely clear what causes these shape changes, but  
they apparently are caused by surface tension, charges  
and/or drying effects.

The spinning technique causes the preferred  
25 monolayer of red blood cells to be formed on the slide  
with the red blood cells separated, that is, spaced  
from each other. Should such a slide be dipped into a  
fixing or staining liquid before the cells are dry,  
the red blood cells would be washed from the slide.  
30 If the red blood cells are fixed in solution, prior to  
deposition on the slide, the fixation causes them to  
cluster together such that a monolayer of separated  
cells is not obtained. Thus, it is desirable to fix  
the cells during the period after monolayer formation,  
35 but prior to drying.

Accordingly, a general object of the invention

is to provide a method for fixing cell morphology of cells on a slide after the monolayer formation, but prior to the drying of the cells on the slide to preserve their identifying features for later analysis.

In one particular aspect the present invention provides a method for preparing a blood specimen having a monolayer of separated red blood cells on a slide suitable for automated analysis, the method comprising the steps of: spinning a sample on a slide about an axis perpendicular to the support for a short  
10 period of time to develop a satisfactory monolayer within an enclosed chamber to separate the red blood cells and to throw excess red blood cells radially outwardly from the slide to form an evenly distributed monolayer of red blood cells, subjecting the monolayer of red blood cells on the slide to a fixing agent for a fixing period subsequent to spinning and prior to drying to fix the natural shapes thereof before the red blood cells flatten during drying on the slide, and drying the fixed red blood cells for later automated analysis.

Other objects and advantages of the invention will  
20 become apparent from the detailed description taken in connection with the accompanying drawing in which:

Figure 1 is an enlarged diagrammatic top view of a microscope slide with a monolayer of red blood cells having central pallors and a red blood cell lacking a central pallor;

Figure 2 is a side elevational view of the slide cells shown in Figure 1;

Figure 3 is a perspective view of an apparatus, in an open position, embodying various features of the invention; and

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Figure 4 is a perspective view of an apparatus, in a closed position, embodying various features of the invention.

Generally, in accordance with the method of the present invention, a quantity of blood sample is placed upon a microscope slide which is then spun by a centrifugal spinner. The spinning action of the slide throws all but a monolayer of the blood from the slide. Before the sample has dried to the extent that red cells would normally be distorted by such drying, a suitable fixing agent is applied to the monolayer to  
10 preserve the shapes of the red cells so that further drying does not adversely change the cell morphology to the extent of losing the central pallor configurations.

Referring to Figure 3, an apparatus for practicing the disclosed method comprises a centrifugal spinner 10 including a housing 12 defining a top-opening interior chamber 14 within which there is disposed a platen 16 rotatably mounted on the end of a shaft 18. The platen

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receives a slide 20 on the upper horizontal flat surface of the platen for spinning of the slide in a horizontal plane. The slide 20 is releasably held in position on the platen by appropriate means 22 such as lugs or the like that are spaced apart on the platen surface to receive the slide. The shaft 18 is driven by motor means (not shown) with the rate and duration of spinning being controlled by conventional means well known in the art. Suitable spinners are commercially available, and may be of the kind shown in U.S. Patent Nos. 3,853,092 and 3,906,890.

In accordance with one embodiment of the disclosed invention, the top opening of the chamber of the spinner is closable as by a covering hood 24 adapted to rest on the housing 12 in position over the top opening. In the depicted embodiments, the hood 24 is connected in communication, as by a conduit 26, with a source of vapororous fixing agent, such as a canister 28 containing the fixing agent. A valve means 30 interposed in the conduit 26 intermediate its opposite ends provides control over the flow of vapor between the source and the interior chamber 14 of the spinner. Herein, the valve 30 comprises a semi-circular valve plate 31 pivoted at the center by a pin 33 fixed on the cover hood. The valve plate is pivoted to cover or uncover the discharge orifice of the conduit 26 when an upstanding handle pin 35 is gripped and swung in an arc. To the end of urging the flow of vapor to the chamber, the pressure within the canister is maintained at a higher level than the pressure within the spinner chamber, such as by a fan 32 mounted within the canister 28 or by an air pump 34 supplying air to the canister.

As noted above, in preparing a blood sample for analysis, a quantity of the blood sample 36 is placed upon the slide 20 which is then mounted upon the spinner platen 16 disposed within the chamber 14. The slide is

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spun at a rate and for a period of time necessary to develop an evenly distributed monolayer of red blood cells upon the surface of the slide. Commonly, the spinning rate is held constant, at a rate between 4,000 and 10,000 rpm with the actual period of spinning being relatively short, e.g., less than one second 2.5 seconds, as is required to develop a satisfactory monolayer.

As disclosed in an article by James W. Bacus, "Erythrocyte Morphology and Centrifugal 'Spinner'

10 Blood Film Preparations", The Journal of Histochemistry and Cytochemistry, 22:7:506-516, 1974, the blood samples are preferably diluted to adjust their plasma viscosities such that the sample may be spun for constant spin time and at a constant speed to provide the monolayer of cells  
15 having good separation and central pallor development. By way of example, a thoroughly mixed sample of whole blood was diluted with a serum albumin solution of 1.3 relative viscosity to adjust the hematocrit of each sample to 18%. The viscosity was relative to H<sub>2</sub>O as 1.0  
20 and a typical range of relative viscosities for blood plasma is 1.2-1.8. Alternatively, a standard isotonic saline solution in a convenient ration, e.g., 1:1 may be used as a diluent for most bloods. As is recognized in the art, if the spinning period is too short, the cells  
25 are clustered together and thus impossible to isolate and classify individually. If the spinning period is too long, there is an undue distortion of the cell shapes.

Rather than adjusting the plasma viscosity and using a constant spin time, as preferred, spin apparatus  
30 may be used which controls the spinning period. One of these is disclosed in Patent No. 3,827,805 and employs a light beam which passes through the slide and sample during spinning. By measuring the degree of scattering of the beam caused by the blood cells, spinning may be  
35 stopped when a predetermined cell distribution is

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achieved. Another apparatus for controlling the spinning period is disclosed in Patent No. 3,906,890. Using this apparatus, the operator determines the approximate blood cell concentration of the sample and sets the spinning  
5 time in accordance therewith. The present invention may be used with either of these patented apparatuses, but is not limited to either such apparatus.

Desirably, the monolayer of red blood cells on the side is fixed and dried to the degree that permits  
10 mechanical handling of the slide without disruption or dislocation of the cells. Typically, a monolayer of blood on a slide air dries within 10 to 15 seconds after spinning is stopped, the time varying with humidity and other conditions. Heretofore, drying of the monolayer,  
15 as noted above, resulted in deleterious distortion of the red blood cells with resultant lack of identification of cells with central pallor and false identification of the misshapen cells. By way of example, red blood cells 40 and 42 are illustrated in solid lines in FIGURE 1 as having a cen-  
20 tral pallor area 44, which is fixed by a fixing agent indicated by arrows in FIGURE 2, to fix the central pallor against the loss thereof during a subsequent drying of the cells. Without fixing the cells 40 and 42 prior to drying, the cells flatten and assume the  
25 flattened shapes 40a and 42a, as shown in dotted lines in FIGURE 2. The flattened cells 42a and 40a having a lost or severely distorted central pallor region are found to be difficult to distinguish from a true spherocyte cell 46, which never had a central pallor, as seen in  
30 FIGURE 1, by automated analysis equipment.

In accordance with the disclosed method, it has been found that the red blood cells can be dried adequately without alteration of the cell morphology by  
fixing the red blood cells during the course of or im-  
35 mediately after the spinning, when the cells have attained a monolayer status with proper dispersion of the

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cells, but prior to the time when the drying process has progressed to the extent that the cell morphology is unacceptably altered. Such fixation has been found to set the physical geometry of the cells whereby the drying of the monolayer can proceed without the cell distortion noted in the prior art. In the preferred embodiment, the fixation step is performed when the monolayer has been established and the spinning force has been removed, so that the fixation occurs before too much drying has occurred. This is accomplished in accordance with the present disclosure by introducing into the chamber in which the slide is spun a fixing agent that is in the form of a vapor, so that its presence causes no ill effect upon the blood sample. This fixation step substantially sets the cell morphology.

Fixing of the cells prior to spinning has been found to prevent the dispersion of the cells in the desired monolayer, resulting rather in clumping or stringing of the cells when they subsequently are spun. Also, the introduction of a liquid fixing agent onto the slide, after spinning, but before drying, has been found to disrupt the cell dispersion, even to the point of washing the blood sample from the spinning slide.

One fixing agent which has proven effective is formaldehyde, a gas at normal room temperatures. In the depicted apparatus, formalin (an aqueous solution of between about 37% and 50% formaldehyde and about 15% methanol, by volume), is stored in the canister 28. The formaldehyde escapes from the solution and is conveyed to the chamber 14 of the spinner 10 by the conduit 26. Alternatively, the slide 20 with the monolayer of blood thereon can be quickly removed from the spinner chamber and placed in an atmosphere of vaporous formaldehyde. This latter step is less desirable in view of the relatively short time, i.e., about 5 seconds, available with-

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in which the transfer must be made if the fixation is to take effect before deleterious drying occurs. Further, the "closed" system in the depicted embodiment is preferred as an aid in controlling the vaporous formaldehyde which is toxic at levels greater than 5 ppm and creates a risk at concentrated levels. Exposure of the monolayer for about five minutes to an atmosphere of vaporous formaldehyde provides the desired fixation of the red blood cells.

10           When the blood sample is spun, the vast majority of the sample is spun from the slide to the interior walls of the spinner housing 12. This blood may contain a variety of harmful organisms, so that in some spinners, a liquid (usually water) is cascaded over the interior  
15 walls of the housing 12 during spinning to serve as a safety seal against escape of such organisms from the chamber. The flying blood is thus washed away through a drain in the bottom of the spinning compartment. One method of practicing the present invention includes  
20 replacing this cascading water with formalin. Thus, formaldehyde vapors are produced in the spinner from the timespinning commences. In this method, the spinning and establishment of a monolayer occurs sufficiently rapidly, i.e., within about 0.5 seconds, so that even  
25 though the vaporous formaldehyde is initially present in the spinning chamber, fixation does not occur until after the cells have been dispersed into the monolayer.

After the sample has been fixed and dried, the slide 20 is removed from the spinner or fixing chamber  
30 and analyzed by automated means. Red blood cells 40 and 42, fixed in the manner described herein, have been found to retain their characteristic central pallors 44 and other physical characteristics such that the samples so prepared provide a true and accurate indication of the  
35 morphology of the cells when analyzed using automated analyzers.

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While a preferred fixing agent of formaldehyde has been used effectively, it is expected that other, methyl, ethyl or short-chain alcohols may be used in lieu of formaldehyde, or that other fixations, such as heating, for example, by microwaves, or other physical or chemical means may be used. Therefore, the present invention is not to be construed as limited to any particular fixing agent.

While a preferred embodiment has been shown and described, it will be understood that there is no intent to limit the invention by such disclosure, but rather, it is intended to cover all modifications and alternative constructions falling within the spirit and scope of the invention as defined in the appended claims.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method for preparing a blood specimen having a monolayer of separated red blood cells on a slide suitable for automated analysis, said method comprising the steps of:

spinning a sample on a slide about an axis perpendicular to the support for a short period of time to develop a satisfactory monolayer within an enclosed chamber to separate the red blood cells and to throw excess red blood cells radially outwardly from the slide to form an evenly distributed monolayer of red blood cells, subjecting said monolayer of red blood cells on said slide to a fixing agent for a fixing period subsequent to spinning and prior to drying to fix the natural shapes thereof before the red blood cells flatten during drying on the slide, and drying the fixed red blood cells for later automated analysis.

2. A method in accordance with claim 1 in which a sample of red blood cells is distributed into a monolayer, the improvement including the further step of generating a vapor of a fixing agent and subjecting the cells to the vaporous fixing agent to fix the central pallors of the red blood cells to retain their characteristic shape and size.

3. The method of claim 2 wherein said vaporous fixing agent is vaporous formaldehyde.

4. The method of claim 1 and including the step of removing said slide and monolayer thereon from said spinning chamber after said spinning step and placing said slide and monolayer in a fixing chamber for said fixing period prior to drying said monolayer.
5. A method of preparing dried and separated red blood cells on a support for a later automated analysis comprising the steps of:
- placing a plurality of wet and unseparated blood cells on an upper surface of a support,
  - spinning the wet and unseparated red blood cells about an axis substantially normal to said support surface for a period of time to develop a satisfactory monolayer and to separate the blood cells on the support and to throw excess cells radially outwardly from the support to form a monolayer of wet and separated red blood cells on said support,
  - stopping the spinning and before the blood cells begin to dry and to substantially change their shape, as occurs in a period of about 15 seconds or less when exposed to ambient atmosphere, and fixing the wet red blood cells with a fixing agent for a fixing period of substantially longer duration, and
  - subsequently drying the fixed wet red blood cells on said support to provide a monolayer of separated and fixed dried cells on said support for a later automated analysis.
6. A method in accordance with claim 5 including the step of fixing the wet cells by exposing the wet blood cells to vaporous formaldehyde for a fixing period of about five minutes.



7. A method of preparing dried and separated red blood cells on a support for a later automated analysis comprising the steps of:

placing a plurality of wet and unseparated red blood cells on an upper surface of a support,

spinning the wet and unseparated red blood cells about an axis substantially normal to said support surface for a period of time to develop a satisfactory monolayer and to separate the red blood cells on the support and to throw excess cells radially outwardly from the support to form a monolayer of wet and separated red blood cells on said support,

stopping the spinning and before the red blood cells begin to dry and to substantially change their shape, as occurs in a period of about 15 seconds or less when exposed to ambient atmosphere, exposing the wet red blood cells with central pallors to a vaporous fixing agent and fixing the central pallors within about 15 minutes or less, and

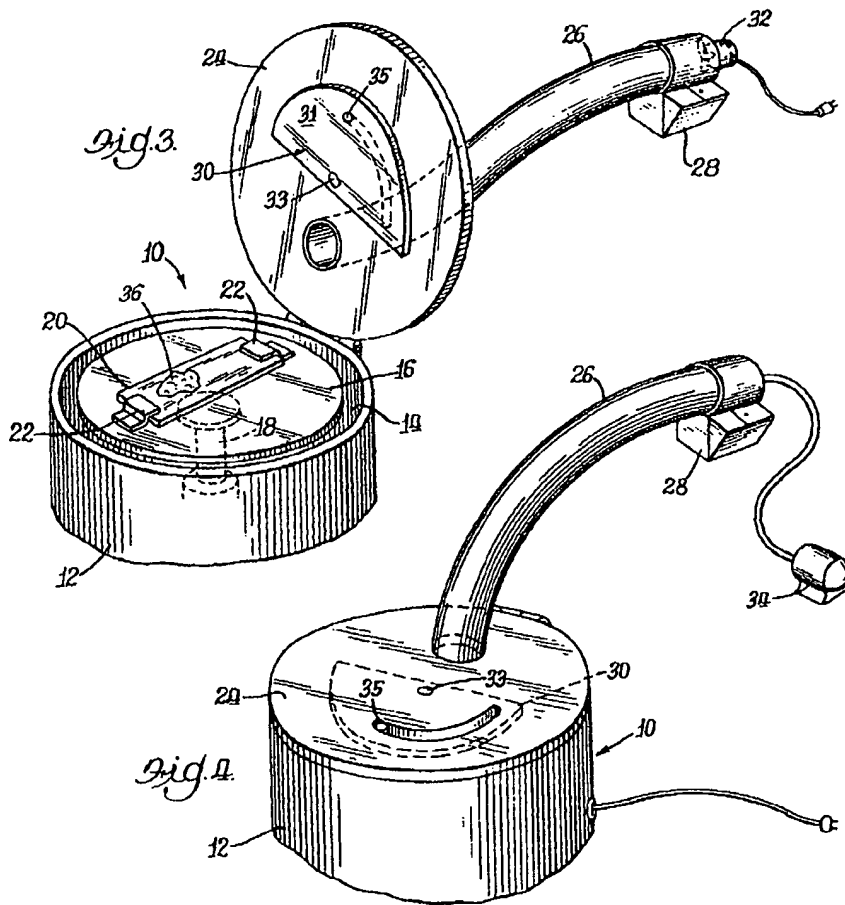
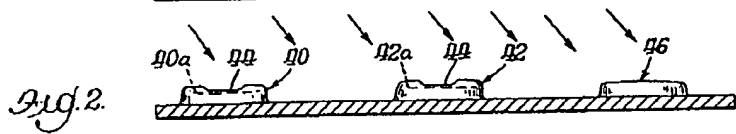
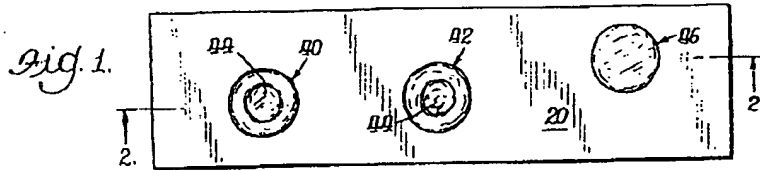
subsequently drying the fixed wet red blood cells on said support to provide a monolayer of separated and fixed dried cells having fixed central pallors on said support for a later automated analysis.

8. An apparatus for practicing the method of claim 1 comprising housing means defining a spinning chamber, rotatable platen means disposed substantially horizontally within said chamber and adapted to receive a support having deposited thereon the wet cells, drive means adapted to rotate said platen means, and means controlling the period of actuation of said

drive means to dispose said wet blood cells in substantially a monolayer on said support, and means for introducing a vaporous fixing agent to said wet cells following their dispersal into substantially a monolayer on said support and prior to their drying and experiencing shape changes.

9. An apparatus in accordance with claim 8 wherein said spinning chamber housing includes interior walls and said means for introducing said vaporous fixing agent comprises means for cascading a safety seal liquid over said interior walls during rotation of said platen means, said liquid releasing said vaporous fixing agent within said spinning chamber.

10. An apparatus in accordance with claim 8 in which a conduit means conveys a vaporous fixing agent into the chamber from an external source to fix the wet cells while still in the spinning chamber.



*alex C. W. Harris*

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